

**What is Claimed is:**

1. An immunogenic composition comprising a cholera holotoxin (CT) and an antigen covalently associated with the CT, wherein the CT comprises an A subunit (CT-A ) having a mutation of at least amino acid residue 29 of SEQ ID NO:2, wherein the mutation is not an aspartic acid, wherein the CT increases immunogenicity of the antigen.  
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2. The composition of claim 1, wherein the CT is further defined as having reduced toxicity relative to a CT comprising a wild-type CT-A.  
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3. The composition of claim 1, wherein the CT-A is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or a degenerate variant thereof, wherein the nucleotide sequence has a genetic modification of at least codon 29 of SEQ ID NO:1.  
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4. The composition of claim 1, wherein residue 29 of SEQ ID NO:2 is an amino acid selected from the group consisting of Ala, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp and Tyr.  
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5. The composition of claim 4, wherein residue 29 is a His residue.
6. The composition of claim 1, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.  
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7. The composition of claim 1, further comprising one or more additional covalently associated antigens selected from the group consisting of a

- polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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8. The composition of claim 1, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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9. The composition of claim 1, further comprising one or more adjuvants.
10. The composition of claim 9, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-7, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .
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11. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.
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12. An immunogenic composition comprising a CT and an antigen covalently associated with the CT, wherein the CT comprises one or more mutations in the CT-A, wherein the CT increases immunogenicity of the antigen.
- 5 13. The composition of claim 12, wherein the CT is further defined as having reduced toxicity relative to a CT comprising a wild-type CT-A.
14. The composition of claim 12, wherein the CT-A comprises an amino acid sequence of SEQ ID NO:2.
- 10 15. The composition of claim 12, wherein the CT-A is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or a degenerate variant thereof.
- 15 16. The composition of claim 14, wherein the one or more mutations are selected from the group consisting of Arg-7, Asp-9, Arg-11, Ile-16, Arg-25, Glu-29, Trp-30, His-44, Val-53, Ser-63, Ser-68, His-70, Val-72, Val-97, Tyr-104, Pro-106, Ser-109, Glu-112 and Arg-192, wherein Glu-29 is not mutated to Asp-29.
- 20 17. The composition of claim 16, wherein one mutation is at Glu-29, wherein the mutation at Glu-29 is not Asp-29.
18. The composition of claim 17, wherein Glu-29 is mutated to a His-29 residue.
- 25 19. The composition of claim 16, wherein one or more mutations is a double mutation at Ile-16 and Ser-68.
20. The composition of claim 16, wherein one or more mutations is a double mutation at Ser-68 and Val-72.
- 30 21. The composition of claim 19, wherein Ile-16 is mutated to Ala-16 and Ser-68 is mutated to Tyr-68.

22. The composition of claim 20, wherein Ser-68 is mutated to Tyr-68 and Val-72 is mutated to Tyr-72.
- 5 23. The composition of claim 14, wherein one or more mutations is an amino acid insertion at amino acid position 49.
24. The composition of claim 14, wherein one or more mutations is an amino acid insertion at amino acid position 36 and an insertion at amino acid position 37.
- 10 25. The composition of claim 14, wherein one or more mutations is an amino acid substitution at amino acid position 30, an amino acid insertion at amino acid position 31 and an insertion at amino acid position 32.
- 15 26. The composition of claim 23, wherein a histidine amino acid is inserted at amino acid position 49 between the wild-type amino acid positions 48 and 49.
27. The composition of claim 24, wherein the amino acids glycine and proline are inserted in the amino acid positions 36 and 37 between the wild-type amino acid positions 34 and 35.
- 20 28. The composition of claim 25, wherein the mutation at amino acid position 30 is a tryptophan and alanine and a histidine are inserted in the amino acid positions 31 and 32 between the wild-type amino acid positions 30 and 31.
- 25 29. The composition of claim 12, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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30. The composition of claim 12, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
31. The composition of claim 12, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
32. The composition of claim 12, further comprising one or more adjuvants.
33. The composition of claim 32, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .
34. The composition of claim 12, further comprising a pharmaceutically acceptable carrier.

35. An immunogenic composition comprising an *Escherichia coli* heat labile toxin (LT) and an antigen covalently associated with the LT, wherein the LT increases immunogenicity of the antigen.
- 5 36. The composition of claim 35, wherein the LT is further defined as having one or more mutations in the LT-A subunit.
37. The composition of claim 36, wherein the one or more mutations are selected from the group consisting of Val-53, Ser-63, Ala-72, Val-97, Tyr-104, Pro-106 and Arg-192.
- 10 38. The composition of claim 37, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
- 15 39. The composition of claim 37, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
- 20 40. The composition of claim 37, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein
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conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

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41. The composition of claim 37, further comprising one or more adjuvants.

42. The composition of claim 41, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-7, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .

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43. The composition of claim 37, further comprising a pharmaceutically acceptable carrier.

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44. An immunogenic composition comprising a pertussis toxin (PT) and an antigen covalently associated with the PT, wherein the PT increases immunogenicity of the antigen.

25 45. The composition of claim 44, wherein the PT is further defined as having one or more mutations in the PT-A subunit.

46. The composition of claim 44, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a

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lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

- 5 47. The composition of claim 44, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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- 15 48. The composition of claim 44, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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49. The composition of claim 44, further comprising one or more adjuvants.
- 25 50. The composition of claim 49, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-7, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .
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51. The composition of claim 44, further comprising a pharmaceutically acceptable carrier.
- 5 52. A method of immunizing a mammalian host comprising administering to the host an immunogenic amount of a composition comprising a cholera holotoxin (CT) and an antigen covalently associated with the CT, wherein the CT comprises an A subunit (CT-A ) having a mutation of at least amino acid residue 29 of SEQ ID NO:2, wherein the mutation is not an aspartic acid, wherein the CT increases immunogenicity of the antigen.
- 10 53. The method of claim 52, wherein the CT is further defined as having reduced toxicity relative to a CT comprising a wild-type CT-A.
- 15 54. The method of claim 52, wherein the CT-A is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or a degenerate variant thereof, wherein the nucleotide sequence has a genetic modification of at least codon 29 of SEQ ID NO:1.
- 20 55. The method of claim 52, wherein residue 29 of SEQ ID NO:2 is an amino acid selected from the group consisting of Ala, Cys, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp and Tyr.
- 25 56. The method of claim 55, wherein residue 29 is a His residue.
- 30 57. The method of claim 52, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

58. The method of claim 52, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
59. The method of claim 52, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
60. The method of claim 52, further comprising one or more adjuvants.
61. The method of claim 60, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-7, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .
62. The method of claim 52, further comprising a pharmaceutically acceptable carrier.

- 5 63. A method of immunizing a mammalian host comprising administering to the host an immunogenic amount of a composition comprising a CT and an antigen covalently associated with the CT, wherein the CT comprises one or more mutations in the CT-A, wherein the CT increases immunogenicity of the antigen.
64. The method of claim 63, wherein the CT is further defined as having reduced toxicity relative to a CT comprising a wild-type CT-A.
- 10 65. The method of claim 63, wherein the CT-A comprises an amino acid sequence of SEQ ID NO:2.
- 15 66. The method of claim 63, wherein the CT-A is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO:1 or a degenerate variant thereof.
- 20 67. The method of claim 65, wherein the one or more mutations are selected from the group consisting of Arg-7, Asp-9, Arg-11, Ile-16, Arg-25, Glu-29, Trp-30, His-44, Val-53, Ser-63, Ser-68, His-70, Val-72, Val-97, Tyr-104, Pro-106, Ser-109, Glu-112 and Arg-192, wherein Glu-29 is not mutated to Asp-29.
- 25 68. The method of claim 67, wherein one mutation is at Glu-29, wherein the mutation at Glu-29 is not Asp-29.
69. The method of claim 68, wherein Glu-29 is mutated to a His-29 residue.
70. The method of claim 67, wherein one or more mutations is a double mutation at Ile-16 and Ser-68.
- 30 71. The method of claim 67, wherein one or more mutations is a double mutation at Ser-68 and Val-72.

72. The method of claim 70, wherein Ile-16 is mutated to Ala-16 and Ser-68 is mutated to Tyr-68.
- 5 73. The method of claim 71, wherein Ser-68 is mutated to Tyr-68 and Val-72 is mutated to Tyr-72.
74. The method of claim 65, wherein one or more mutations is an amino acid insertion at amino acid position 49.
- 10 75. The method of claim 65, wherein one or more mutations is an amino acid insertion at amino acid position 36 and an insertion at amino acid position 37.
- 15 76. The method of claim 65, wherein one or more mutations is an amino acid substitution at amino acid position 30, an amino acid insertion at amino acid position 31 and an insertion at amino acid position 32.
77. The method of claim 74, wherein a histidine amino acid is inserted at amino acid position 49 between the wild-type amino acid positions 48 and 49.
- 20 78. The method of claim 75, wherein the amino acids glycine and proline are inserted in the amino acid positions 36 and 37 between the wild-type amino acid positions 34 and 35.
- 25 79. The method of claim 76, wherein the mutation at amino acid position 30 is a tryptophan and alanine and a histidine are inserted in the amino acid positions 31 and 32 between the wild-type amino acid positions 30 and 31.
- 30 80. The method of claim 63, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a

lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

- 5 81. The method of claim 63, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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- 15 82. The method of claim 63, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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83. The method of claim 63, further comprising one or more adjuvants.
- 25 84. The method of claim 83, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-7, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .
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85. The method of claim 63, further comprising a pharmaceutically acceptable carrier.
- 5 86. A method of immunizing a mammalian host comprising administering to the host an immunogenic amount of a composition comprising an *Escherichia coli* heat labile toxin (LT) and an antigen covalently associated with the LT, wherein the LT increases immunogenicity of the antigen.
- 10 87. The method of claim 86, wherein the LT is further defined as having one or more mutations in the LT-A subunit.
- 15 88. The method of claim 87, wherein the one or more mutations are selected from the group consisting of Val-53, Ser-63, Ala-72, Val-97, Tyr-104, Pro-106 and Arg-192.
- 20 89. The method of claim 88, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
- 25 90. The method of claim 88, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
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91. The method of claim 88, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.
92. The method of claim 88, further comprising one or more adjuvants.
93. The method of claim 92, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A); a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .
94. The method of claim 88, further comprising a pharmaceutically acceptable carrier.
95. A method of immunizing a mammalian host comprising administering to the host an immunogenic amount of a composition comprising a pertussis toxin (PT) and an antigen covalently associated with the PT, wherein the PT increases immunogenicity of the antigen.
96. The composition of claim 95, wherein the antigen is selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a

peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

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97. The composition of claim 95, further comprising one or more additional covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

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98. The composition of claim 95, further comprising one or more additional non-covalently associated antigens selected from the group consisting of a polypeptide, a polypeptide fragment, a carbohydrate, an oligosaccharide, a lipid, a lipooligosaccharide, a polysaccharide, an oligosaccharide-protein conjugate, a polysaccharide-protein conjugate, a peptide-protein conjugate, an oligosaccharide-peptide conjugate, a polysaccharide-peptide conjugate, a protein-protein conjugate, a lipooligosaccharide-protein conjugate and a polysaccharide-protein conjugate.

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99. The composition of claim 95, further comprising one or more adjuvants.

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100. The composition of claim 99, wherein one or more adjuvants are selected from the group consisting of GM-CSF, 529SE, IL-12, aluminum phosphate, aluminum hydroxide, *Mycobacterium tuberculosis*, *Bordetella pertussis*, bacterial lipopolysaccharides, aminoalkyl glucosamine phosphate compounds, MPL (3-O-deacylated monophosphoryl lipid A), a polypeptide, Quil A, QS-21, a pertussis toxin (PT), an *E. coli* heat-labile toxin (LT), IL-1  $\alpha$ , IL-1  $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-14, IL-15, IL-16, IL-7,

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IL-18, interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , granulocyte colony stimulating factor, tumor necrosis factor  $\alpha$  and tumor necrosis factor  $\beta$ .

- 5      101. The composition of claim 95, further comprising a pharmaceutically acceptable carrier.